Gibson Assembly (E5510 NEB version)

Introduction

Overview of Gibson Assembly Cloning Kit Protocol:
Design primers to amplify fragments (and/or vector) with appropriate overlaps
PCR amplify fragments using a high-fidelity DNA polymerase.
Prepare linearized vector by PCR amplification using a high-fidelity DNA polymerase or by restriction digestion.
Confirm and determine concentration of fragments and linearized vector using agarose gel electrophoresis, a NanoDrop™ instrument or other method.
Add fragments and linearized vector to Gibson Assembly Master Mix and incubate at 50°C for 15 minutes to 1 hour, depending on number of fragments being assembled.
Transform into NEB 5-alpha Competent E. coli (provided) or use directly in other applications.

Optimal Quantities

NEB recommends a total of 0.02–0.5 pmols of DNA fragments when 1 or 2 fragments are being assembled into a vector and 0.2–1.0 pmoles of DNA fragments when 4–6 fragments are being assembled. Efficiency of assembly decreases as the number or length of fragments increases. To calculate the number of pmols of each fragment for optimal assembly, based on fragment length and weight, we recommend using NEB's online tool, NEBioCalculator , or using the following formula:

pmols = (weight in ng) x 1,000 / (base pairs x 650 daltons)

50 ng of 5000 bp dsDNA is about 0.015 pmols.

50 ng of 500 bp dsDNA is about 0.15 pmols.

The mass of each fragment can be measured using the NanoDrop instrument, absorbance at 260 nm or estimated from agarose gel electrophoresis followed by ethidium bromide staining.

Optimized cloning efficiency is 50–100 ng of vectors with 2–3 fold of excess inserts. Use 5 times more of inserts if size is less than 200 bps. Total volume of unpurified PCR fragments in Gibson Assembly reaction should not exceed 20%.

Materials

- DNA fragments
- Gibson Assembly Master Mix
- Deionized H2O
- Ice
- Thermocycler

Procedure

Step 1

1. Set up the following reaction on ice:

Table1

	A	B	C	D
1		**2-3 fragment assembly**	**4-6 fragment assembly**	**Positive contr**
2	**Total amount of fragments**	0.02 - 0.5 pmol X µl	0.2 - 1 pmol X µl	10 µl
3	**Gibson Assembly Master Mix (2x)**	10 µl	10 µl	10 µl
4	**Deionized H2O**	10 - X µl	10 - X µl	
5	**Total volume**	20 µl	20 µl	20 µl

Step 2

2. Incubate samples in a thermocycler at 50°C for 15 minutes when 2 or 3 fragments are being assembled or 60 minutes when 4-6 fragments are being assembled.

Step 3

3. Store samples on ice or at –20°C for subsequent transformation.

Step 4

4. Transform NEB 5-alpha Competent E. coli cells (provided with the kit) with 2 µl of the assembly reaction, following the transformation protocol.

Transformation

5. 1. Thaw chemically competent cells on ice.
   2. Add 2 µl of the chilled assembly product to the competent cells. Mix gently by pipetting up and down or by flicking the tube 4–5 times. Do not vortex.
   3. Place the mixture on ice for 30 minutes. Do not mix.
   4. Heat shock at 42°C for 30 seconds. Do not mix.
   5. Transfer tubes to ice for 2 minutes.
   6. Add 950 µl of room-temperature SOC media to the tube.
   7. Incubate the tube at 37°C for 60 minutes. Shake vigorously (250 rpm) or rotate.
   8. Warm selection plates to 37°C.
   9. Spread 100 µl of the cells onto the selection plates. Use Amp plates for positive control sample.
   10. Incubate overnight at 37°C